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THE TRANSMISSION OF LEISHMANIA
INFECTIONS

C.M.WENYON

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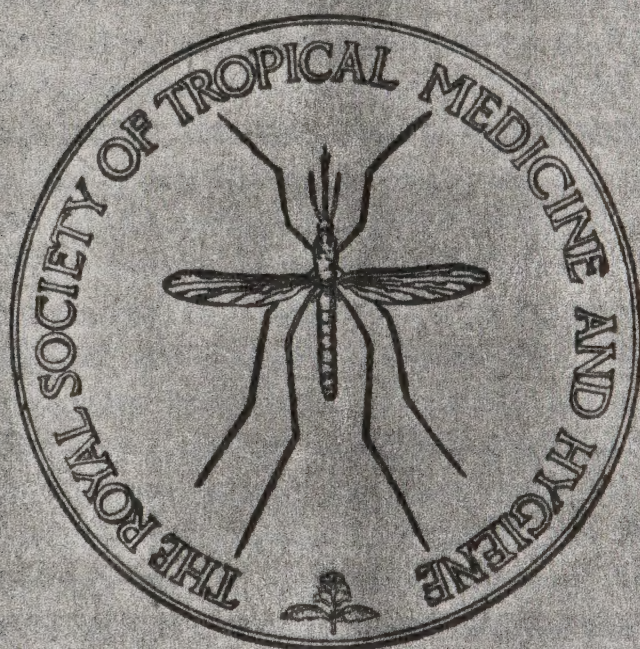
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PAPER.

THE TRANSMISSION OF LEISHMANIA INFECTIONS.

A REVIEW.

BY

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Since the observation of KNOWLES, NAPIER and SMITH (1924) in Calcutta that the parasite of kala-azar taken up by the sandfly *Phlebotomus argentipes* when feeding on cases of the disease develops into flagellates of the leptomonas type and that these multiply to produce a massive infection of the mid-gut, a result which was quickly confirmed by CHRISTOPHERS, SHORTT and BARRAUD (1925) in Assam, research to discover the mode of transmission has been almost entirely occupied with experiments with this insect and allied species. Similarly since SERGENT, PARROT, DONATIEN and BÉGUET (1921) reported the production in Algiers of oriental sore in a man inoculated in the skin with an emulsion of *P. papatasi* collected in Biskra, and the observations of ADLER and THEODOR (1925) in Palestine that a leptomonas found by them in wild *P. papatasi* caused oriental sore when inoculated in the skin and that laboratory-bred sandflies acquired a massive infection of leptomonas when fed on the sore produced, practically all investigation on the method of spread of this disease has concerned itself with sandflies. Thus in the cases of both kala-azar and oriental sore during the past ten years research in connection with transmission has occupied itself chiefly with sandflies of the genus *Phlebotomus*. Though, during this period, very interesting results have been obtained, all of which appear to indicate a specific relationship between sandflies and leishmania, there was the difficulty that actual transmission by the bite could not be effected even to the Chinese hamster which was shown by SMYLY and YOUNG (1924) to be highly susceptible to inoculation. In one case, however, ADLER and THEODOR (1929a) appear to have transmitted oriental sore to man by the bite of *P. papatasi* in an experiment which, however, they do not claim to be conclusive, while quite recently SHORTT, SMITH, SWAMINATH and KRISHNAN (1931) report the production of kala-azar in a hamster on which had fed, during a period of seventeen months, twelve dozen *P. argentipes*, the majority of which must have been infected. Working in Tashkent, CHODUKIN, SOFIEFF, SCHEVTSCHENKO and RADOWILOVSKIJ (1931) state that a healthy dog kept at one end of a fly-proof cage, at the other end of which was a dog suffering from cutaneous

leishmaniasis, contracted the infection in five months after sandflies had been repeatedly introduced during the course of the first three months of the experiment. Apart from these instances, in spite of very intensive research involving thousands of sandflies, there has been complete failure to transmit kala-azar or oriental sore by these insects.

On account of this failure there has been a tendency to revive the older theories or to introduce new ones which it will be well to examine. When first discovered there was considerable doubt as to the real nature of *Leishmania donovani* and *L. tropica* but the announcement of ROGERS (1904) that the parasite of kala-azar developed into flagellates of the herpetomonas or leptomonas type in culture media, an observation extended to the parasite of oriental sore by NICOLLE (1908), established their affinities. Trypanosomes were known to be transmitted by tsetse flies and it seemed natural to suppose that the flagellates of kala-azar and oriental sore would similarly have some insect vector. Flagellates of the leptomonas type were known to be common inhabitants of the intestine of insects and it was to be expected that in an insect vector the leishmania would develop into leptomonas, as they did in the culture tube. The flagellate stage of leishmania is such an important one that it seems safe to conclude that any theory of transmission that does not give it a place cannot be correct.

DIRECT INFECTION.

Though the majority of observers were convinced that some blood-sucking insect was the vector, direct infection from man to man by the contaminative method was held by some to be a possibility and certain facts appeared to support this hypothesis. It was known that oriental sore could be inoculated from man to man and in Bagdad it was a common practice to inoculate on some unexposed part of the body to prevent the contraction of unsightly sores and scars on the face. It seems highly probable that having acquired one sore children will auto-inoculate themselves at other places by scratching insect bites or abrasions. House flies have been accused of conveying parasites from one person to another on the proboscis or feet and such a method of infection must seem possible to those who have seen an oriental sore on a child's face covered with flies. If flies convey leishmania, or in fact any other organisms, it seems probable that they do so much less readily by the proboscis or feet than by way of the intestinal tract. The writer (1911a) in Bagdad showed that house flies readily ingest parasites from the surface of an oriental sore and it is probable that within a few minutes they may pass them unaltered in their dejecta on the skin of another individual. Working in Egypt during the war the writer and O'CONNOR (1917) showed that house flies would take up living trichomonas from faeces and deposit them still living and unaltered in their dejecta five minutes later. Though such a method of transmission of leishmania from person to person may take place, it is too precarious a means of transmission to be a natural one. Furthermore,

the parasites will not develop into flagellates in the intestine of the house fly, from which they quickly disappear, so that the theory leaves unexplained the flagellate stage of the organism. In the case of kala-azar contaminative infection seemed a possibility after ARCHIBALD (1914) had shown that monkeys may acquire the disease if fed on the spleen of kala-azar cases, an observation which was confirmed for these or other animals by CORNWALL and LA FRENAIS (1916), KNOWLES, NAPIER and DAS GUPTA (1923), GREIG and CHRISTOPHERS (1925), SHORTT, CRAIGHEAD, SMITH and SWAMINATH (1928) and KHAW (1930, 1931). SHORTT and his co-workers (1928a) also showed that infection could take place by the conjunctival route. In support of such a method of infection ADELHEIM (1924) announced that a healthy mouse acquired infection after being kept for five months with an infected mouse, a similar observation being made in the case of the hamster by SHORTT, CRAIGHEAD, SMITH and SWAMINATH (1930a). Whether such infections are due to ectoparasites or to the ingestion of the excreta of the infected animal is not known. PERRY (1922) found that the villi of the intestine of cases of kala-azar might be packed with macrophages laden with parasites so that escape into the intestine seemed possible if ulceration occurred. This conjecture was proved to be correct by SHORTT, SMITH, D'SILVA and SWAMINATH (1929), who demonstrated leishmania in the blood-stained mucus of a case of kala-azar complicated by dysentery. SHORTT (1923a) and SHORTT, SWAMINATH and SEN (1923) had already shown that leishmania occurred in the urine of kala-azar cases and could be cultivated from the centrifuged sediment. It is known, however, that the parasite will not survive in water and will not develop into a flagellate in any blood-free medium. The contaminative method of infection in kala-azar can only take place if the interval between discharge of parasites and their ingestion is a very short one, while it again leaves unaccounted for the flagellate stage, which practically never occurs in the body of the warm-blooded host and is only present in blood-containing media. It seems that the flagellate stage can have no satisfactory explanation except in association with some insect or other invertebrate.

BLOOD-SUCKING INSECTS.

Possibility of Ingestion of Leishmania.

If now we turn our attention to blood-sucking invertebrates as transmitting agents it has to be assumed that they are able to ingest parasites during the act of feeding. In the case of oriental sore, especially in the pre-ulcerative stage, parasites occur in large numbers just below the thin epidermal covering. Any blood-sucking insect which feeds on the surface of such a sore will ingest parasites. In the case of kala-azar it might not at first sight appear to be so simple, as the parasites are situated in large numbers in the spleen, liver, bone marrow and other internal organs. CHRISTOPHERS (1904, 1904a) first showed that parasites could be found in films of the peripheral blood, an observation

which was repeated by PATTON, DONOVAN and a number of other workers. MAYER and WERNER (1914) and the writer (1914a) almost simultaneously demonstrated that cultures could be obtained from the peripheral blood. Culture from the peripheral blood has now become a procedure of diagnostic importance. ADLER and THEODOR (1931) working in Sicily have recently shown that 0.03 c.cm. of blood is frequently sufficient to yield a culture. In this connection it has also to be noted that CASH and HU (1927) have found that in experimentally infected hamsters enormous numbers of parasites may be found in the skin, the large infected macrophages or clasmocytes forming a layer all over the body. They have also shown that the same condition may occur in human beings. In India BRAMACHARI (1922) first called attention to a condition which he named dermal leishmanoid. In a case of kala-azar which had been treated and had apparently recovered there subsequently developed on the skin nodules resembling those of leprosy; leishmania were demonstrated in these lesions. Since then other similar cases have come to light and it is evident from the work of ACTON and NAPIER (1927) and NAPIER and GUPTA (1930), who refer to the condition as post-kala-azar dermal leishmaniasis, that these cutaneous infections, which in their early stages are represented by small depigmented areas of skin, are fairly common in Bengal and are by no means limited to treated cases of kala-azar. In a recent paper NAPIER and KRISHNAN (1931) conclude that cases of dermal leishmaniasis are becoming common in Bengal and are associated with the development of immunity in the population long exposed to infection. They consider it probable that during epidemic waves, as in Assam, the disease is maintained by the vector, which they assume to be the sandfly, taking up parasites from the blood and producing the same type of infection, while in Bengal there is an increasing tendency for the vector to obtain the parasites from the skin and on transmission to produce the dermal condition alone. ADLER and THEODOR (1931c) have shown that in the Mediterranean area dogs may harbour parasites in large numbers in the skin even when they are difficult to find in the spleen or bone marrow. CHODUKIN and SCHEWTSCHENKO (1928) had already called attention to the presence of cutaneous lesions containing leishmania in cases of canine kala-azar in Tashkent, while DONATIEN, LESTOQUARD and PARROT (1929) in Algeria described a case of canine disease in which there were more numerous parasites in the cutaneous ulcers than in the internal organs. It is clear, therefore, that a blood-sucking insect has every opportunity when feeding on a case of kala-azar of taking up parasites either from the blood or from the skin.

Relation of Leishmania to Insect Leptomonas.

Before discussing blood-sucking insects it will be advisable to consider the parasites of kala-azar and oriental sore and their allies in the light of modern knowledge. As already noted, the leishmania in cultures develop into

flagellates of the leptomonas type, the leishmania being merely rounded non-flagellate forms of these which, it must be remembered, are not resting stages, for they are capable of reproduction like the flagellate forms. Flagellates of the leptomonas type are essentially intestinal parasites of insects and some other invertebrates. The vast majority of them are limited to insects and pass from insect to insect through the agency of rounded leishmania forms discharged in the fæces. Thus fleas, like the dog flea, are liable to infections of this kind. In the hind gut of the flea occur flagellates (*Leptomonas ctenocephali*) either free in the lumen or attached to the wall particularly around the pyloric opening. In the posterior portion of the intestine the flagellates show a tendency to become shorter and finally, losing their flagella, become small round or ovoid bodies having the structure of the parasites of kala-azar and oriental sore. These small bodies of the leishmania type can withstand considerable drying. They escape in the fleas' fæces which, being composed very largely of semi-digested blood, are devoured by the flea larvæ. When, after pupation, such larvæ become adult fleas the leptomonas, having survived the metamorphosis, are found in the intestine. The flagellate thus passes directly from flea to flea through the agency of leishmania forms, probably encysted but in any case resistant, which escape in the fæces. The life history of all insect leptomonas as far as is known is of the same type. It is important to note that such leptomonas in their natural hosts exhibit three features which will serve as a guide as to whether a host is a natural one or not. Firstly, small doses of infective forms will give rise to heavy infections, showing that reproduction progresses without hindrance. Secondly, the infections once established are persistent while thirdly, the flagellates attach themselves to the walls of the intestine and in some cases other organs by their flagellar ends. Knowledge of the leptomonas of insects, due largely to the researches of PATTON in India, strengthened the view that the parasites of kala-azar and oriental sore represented some insect leptomonas.

Inoculation of Insect Leptomonas to Vertebrates.

The resemblance between the naturally occurring leptomonas of insects and the cultural forms of leishmania led to a series of experiments which at one time promised very interesting results. Certain observers attempted to infect laboratory animals, birds, fish, reptiles and amphibia by feeding them or inoculating them with various insect leptomonas. It was claimed not only that infection had occurred in a large percentage of cases but that fatal diseases resembling kala-azar had been produced. Repetition of these experiments by a number of competent observers, however, failed entirely to confirm these findings, so that there was no alternative to the conclusion that the results previously reported were the outcome of some unexplained fallacy.

Leishmania in Animals.

Though the leishmania of kala-azar and oriental sore as parasites of man have been known for a considerable time, and in this connection it may be well to mention that in oriental sore the parasite was first figured and described in 1898 as a protozoon by BOROWSKY, a Russian military surgeon, working in Tashkent, it was some years later that they were demonstrated in animals. Kala-azar in dogs was first described by NICOLLE and COMTE (1908) in Tunis, since when the canine disease has been recorded from the whole of the Mediterranean littoral and islands and from the Trans-Caspian region, while cases have been noted in Dakar by LAFONT and HECKENROTH (1915) and by MARCEL LEGER (1931). The SERGENTS, LOMBARD and QUILICHINI (1912) reported a case of kala-azar in a cat seen in a house near Algiers where a case of human kala-azar occurred. Oriental sore in dogs has been described by a number of observers and recently it has been noted in the dog, cat and bear in Bagdad by CHADWICK, MACHATTIE and MILLS (1927, 1927*a*, 1931, 1931*a*). A single case of generalised leishmania infection of the horse was recorded by RICHARDSON (1926) from Uganda, while in North China YOUNG and HERTIG (1926) reported that out of 4,480 hamsters examined one was found infected. As the parasites of oriental sore and kala-azar are inoculable to monkeys, dogs and other animals it is not surprising that natural infections in susceptible animals occur, but of another nature are the infections which occur naturally in lizards. A parasite (*Leishmania chamaeleonis*) was discovered by BAYON (1915) in the cloaca of *Chamaeleon pumilis* of Robben Island and the writer (1921) found it later in *C. vulgaris* of Egypt. This parasite appears to be limited to the cloaca of the chamaeleon where it occurs as typical leptomonas and leishmania forms. In another lizard of the genus *Anolis* in Martinique MARCEL LEGER (1918) discovered a leptomonas in the blood and also in the intestine. The SERGENTS, LEMAIRE and SENEVET (1914) had previously shown that the gecko *Tarentola mauritanica* of North Africa harboured a leptomonas in the blood but whether the flagellate occurs in the intestine also is not known. It was thought at one time that this gecko might be a reservoir of *L. tropica* of man. Subsequent work, however, has shown that this is not the case. Another leptomonas of the gecko was described by MACKIE, GUPTA and SWAMINATH (1923) from the blood of *Hemidactylus gleadowii* of India. A similar leptomonas was described by STRONG (1924) from the hind gut of a lizard (*Cnemidophorus lemniscus*) of Central America. He supposed that the lizard acquired the infection by eating certain plant bugs which in their turn became infected by sucking the juices of euphorbias, which plants are well known to be liable to leptomonas infection. It must be admitted, however, that this course of events has not been proved experimentally. ADLER and THEODOR (1929) discovered a leptomonas in the blood of the Bagdad gecko *Ceramodactylus doriae*, while DAVID (1929) found one in the blood of the lizard *Agama stellio* in Palestine. In all cases where blood infections of lizards have been demonstrated this

has been done by the culture method alone but in two instances leishmania forms have been found in the blood. SHORTT and SWAMINATH (1928) examining in India the blood of a gecko of the genus *Hemidactylus* from which leptomonas had been cultivated, found leishmania in mononuclear cells. A culture from the liver gave an abundant growth of leptomonas which is evidently identical with the parasite described by MACKIE, GUPTA and SWAMINATH (1923). DAVID (1929) examining the blood of *A. stellio* from which she had cultivated a leptomonas, found a mononuclear cell containing sixteen leishmania. These discoveries afford some justification for the inclusion of the lizard leptomonas in the genus *Leishmania*. As regards these parasites of lizards it seems highly probable that in the case of the chamæleon the cloacal infection is the result of feeding on insects harbouring leptomonas. The same is probably true of the leptomonas of *Anolis* sp. which occurs in the intestine and in the blood, the blood infection having resulted from invasion from the intestine. In other cases the flagellates have been found only in the blood but here again it seems probable that the infection is intestinal in the first place though evidence of this is lacking.

All these observations have a direct bearing on the problem of transmission of kala-azar and oriental sore and make it almost impossible to believe that some insect vector is not involved.

THE BED BUG THEORY.

As remarked above, owing to the almost universal failure to transmit human leishmaniasis, particularly kala-azar, by the bite of the sandfly, some observers have been inclined to revert to older theories. Thus YOUNG and HERTIG (1929) raise the question as to whether the development of leishmania in the sandfly is any indication of a specific relationship between the parasites and these insects, while SHORTT, CRAIGHEAD, SMITH and SWAMINATH (1929), in commenting on their infection of hamsters by feeding them with infected liver and spleen, state that the question of oral infection is re-opened by these results.

The first great impetus to the theory of insect transmission of kala-azar was given by the observation of PATTON (1907) that in the stomach of the bed bug the parasite of kala-azar developed into leptomonas as it did in the culture tube. The writer (1911a) showed that the parasite of oriental sore behaved in a similar manner. As a result of a series of very careful observations and experiments extending over a number of years PATTON became an advocate of the theory of bed bug transmission of kala-azar and oriental sore. There were, however, several points which were difficult to reconcile with what was known of the disease. Oriental sore occurs on exposed parts of the body and it did not seem that bed bugs necessarily selected these places for feeding, while as regards both diseases there was no correlation

between their distribution and that of bed bugs. For instance, kala-azar was common in Calcutta but not in Bombay though bed bugs abounded in both places. Furthermore, the development of the parasite in the bugs did not appear to be of the type one would expect in a natural host. There was no tendency for the parasites to multiply to any extent, the infections were liable to die out and there was no indication that the flagellates attached themselves to the intestinal wall. As demonstrated by PATTON, a second feed of blood was sufficient to clear the intestines of any flagellates which might have developed from previously ingested leishmania. If few parasites were ingested by the bugs few flagellates occurred and massive infections were only encountered when bugs had taken up many organisms, as, for instance, after feeding on rich cultures. Furthermore, the writer (1912) showed that *Trypanosoma lewisi* of the rat would survive and develop to a certain extent in the bed bug though there was no question of it being a host of the trypanosome. For these reasons he became convinced that the stomach of the bed bug was merely playing the part of a culture tube and that the development that took place was no evidence that the bug was a natural host of leishmania. That the organisms which survive and develop in the bug are living and virulent was demonstrated by SHORTT and SWAMINATH (1924) who injected into mice emulsions of bugs fed nine days previously on cases of kala azar. Of a number of mice thus treated one became infected. Similarly YOUNG and HERTIG (1929) proved that leishmania injected into the body cavity of bugs were capable of infecting hamsters by inoculation forty days later. BLACKLOCK and LOURIE (1931) have recently shown that the parasites of kala-azar and oriental sore fed to bed bugs as cultural forms can be cultivated from the dejecta for many days—in the case of *L. tropica* thirty-one days—after ingestion. It is significant that they have shown that the flagellates of the mosquito (*Herpetomonas culicidarum*) can similarly survive for a number of days. PATTON, LA FRENAIS and RAO (1921) had previously shown that certain insect flagellates will remain alive in the stomach of the bug for as long as forty-five days, while SHORTT (1923) has obtained active multiplication of the leptomonas of the flea in the stomach of this insect. As there is no specific relationship between the bed bug and these insect flagellates it seems clear that any multiplication or survival which occurs is merely the result of what may be termed the culture tube-like nature of the stomach of the bug. NICOLLE and ANDERSON (1925) in Tunis made careful experiments in which nearly 1,000 bugs were used in an unsuccessful attempt to transmit kala-azar from dog to dog. SHORTT and SWAMINATH (1925) working in India likewise failed to transmit kala-azar to monkeys through the agency of these insects though they were subjected to repeated bites during the course of one year. It should be mentioned, however, that YOUNG (1927) writing of investigations carried out by himself and HERTIG in China states that transmission to the hamster was effected in one instance by the bed bug. It is curious that in the detailed description of the bed bug experiments YOUNG

and HERTIG (1926*b*) make no mention of this. It is the writer's opinion, therefore, that the bed bug can be dismissed from any theory which attempts to explain the method of spread of kala-azar and oriental sore.

THE DOG TICK THEORY.

The recent observations of BLANC and CAMINOPETROS (1930*a*, 1931) in Athens on the dog tick are closely related, though in this case there is a possibility that transmission may be effected in some cases. They have noted that the larvæ or nymphs of the dog tick which have fed on infected hamsters or naturally infected dogs take up parasites and that these survive the period of moult. Ticks fed as larvæ kept till they have passed through ecdysis to the nymph stage and nymphs similarly fed and kept till they have become adults will infect hamsters if they are ground up and injected. As noted above, it is known that animals can be infected by the ingestion of infected tissues and it is possible that dogs may acquire infection by devouring nymphs or adults which have taken up parasites from an infected dog when they were in the preceding stage of their development. Another parasite of the dog, the leucocytic hæmogregarine *Hepatozoon canis*, is acquired by dogs through eating infected ticks. The writer (1931) has infected dogs in this country by feeding them with ticks sent from Bagdad. He does not agree, however, with the conclusions of BLANC and CAMINOPETROS that because of this survival the dog tick, which rarely fixes itself to human beings, must be regarded as the vector of kala-azar in the Mediterranean area. The mere survival of an organism for a number of days in the alimentary tract cannot be accepted as evidence of a specific relationship between host and parasite in the absence of a special type of development or behaviour.

THE FLEA THEORY.

The discovery of kala-azar in dogs naturally led observers to suspect the dog flea as a possible host for the parasite and the Italian observer BASILE undertook experiments in this connection. Without going into details of his work suffice it to say that he claimed to have followed the development of the parasite in fleas and to have effected transmission by their agency. This work was hampered by the fact that fleas are liable to a natural infection with leptomonas and there is no doubt that BASILE confused the two parasites. The writer (1912) fed clean fleas on an oriental sore and was able to show that the dejecta of the fleas passed within a few minutes of feeding contained unaltered parasites. Though parasites were present in the flea's intestine they showed no signs of development and rapidly disappeared. In connection with canine kala-azar in Malta the writer (1914) conducted an experiment with four dogs sent from London. Two in flea-proof cages were exposed to the bites of fleas taken from a naturally infected dog and two were controls. The flea-bitten dogs died but not from leishmaniasis, but from flea anæmia induced by

the bites of many thousand fleas which had sprung from the four hundred or so introduced into the cage. PEREIRA DA SILVA (1913) and NICOLLE and ANDERSON (1923, 1926) have conducted similar experiments with like results. ADLER has recently informed the writer that in Malta, sandflies can be infected by feeding them on dogs suffering from kala-azar while fleas taken from the same dogs show no signs of infection.

Mosquitoes, culicoides, conorrhinus, lice, house flies and even ancylostomes have received attention without throwing any light on the mystery. It should be noted, however, that in the case of lice taken off infected hamsters YOUNG and HERTIG (1926a) showed that they contained leishmania capable of infecting hamsters on inoculation. Attempts to transmit infection from hamster to hamster by transferring lice from infected to healthy animals failed entirely.

THE SANDFLY THEORY.

As on epidemiological and experimental grounds none of the theories so far considered satisfy the requirements we are left with our only hope, the sandfly, which PRESSAT (1905) and the brothers SERGENT (1905) suspected as being responsible for oriental sore. Before the announcement of KNOWLES, NAPIER and SMITH (1924) which brought suspicion on *P. argentipes* as the vector of kala-azar in India several observations which had a definite bearing on the leishmania sandfly problem were made. The writer (1911) discovered in sandflies in Aleppo a leptomonas which was very probably *L. tropica*, while ACTON (1919) in Mesopotamia drew attention to a correlation which existed between the positions on the body where oriental sore occurred and the sites selected by sandflies for feeding.

Sandfly Experiments.

The next publications dealing with leishmania and sandflies were a series of papers by LAVERAN and FRANCHINI. It was announced by them (1920, 1920a) that they had discovered a leptomonas in *P. papatasi* in Italy, had cultivated it and produced in dogs not only oriental sore but also generalised infections resembling kala-azar. Though neither human nor animal leishmaniasis occurs in Bologna, where the sandflies were collected, it is stated that four out of two hundred examined were infected, an infection rate, which is very much greater than has been found amongst sandflies even in endemic centres of oriental sore and kala-azar. The figures of the flagellates might represent crithidia as well as leptomonas, while there are so many discrepancies in these and other papers which claim the successful inoculation of insect flagellates to vertebrates, observations which, as noted above, have never received confirmation, that it is evident the statements cannot be accepted as correct. There appears to be no alternative to ignoring them entirely. From Algiers, however, came evidence

of a much more definite nature. SERGENT Ed. and Et., PARROT, DONATIEN and BÉGUET (1921) performed a very interesting experiment. Specimens of *P. papatasi* collected in Biskra, an endemic centre of oriental sore, were sent to the Pasteur Institute at Algiers, a three days' journey. These were emulsified in saline and inoculated into the skin of a man by scarification. About two months later a papule had formed at the site of inoculation and this developed into a typical oriental sore in which parasites indistinguishable from *L. tropica* were found. ARAGÃO (1922) in the following year working in Brazil fed sandflies on sores and three days later crushed them in saline and inoculated a dog on the nose with the result that a sore containing leishmania developed. These experiments appeared to bring grave suspicion on the sandfly as a transmitter of oriental sore but as regards kala-azar it was the startling observation of KNOWLES, NAPIER and SMITH (1924) that *P. argentipes* became heavily infected with leptomonas after feeding on cases of kala-azar that directed attention to these insects in the case of the more serious disease. The particular sandfly *P. argentipes* had been selected for experiment because SINTON in his work on the sandflies of India had noted a correlation between the distribution of this species and kala-azar. From this time onwards intensive investigations of sandflies in relation to kala-azar and oriental sore were carried on. The Calcutta observers continued their work on kala-azar at the Calcutta School of Tropical Medicine, CHRISTOPHERS, SHORTT, BARRAUD and others confirmed the results in Assam, YOUNG, HERTIG and later the Royal Society's Commission under PATTON and HINDLE obtained similar results with this disease and sandflies in North China, while with oriental sore ADLER and THEODOR (1925, 1926) in Palestine obtained the same type of development of the parasite in these insects. Furthermore, naturally infected sandflies were reported from Assam and Palestine and ADLER and THEODOR showed that these naturally occurring flagellates inoculated into the skin of man produced oriental sore from which sandflies could again be infected. It seems justifiable to conclude that the emulsion of sandflies inoculated into the skin of a man by the SERGENTS and their co-workers in 1921 and which gave rise to a typical oriental sore must have contained flagellates though these had not been seen by them. Still later PARROT, DONATIEN and LESTOQUARD infected sandflies in Algiers by feeding them on animals inoculated with *L. tropica* and on dogs suffering from kala-azar while ADLER and THEODOR as members of another Royal Society's Commission have recently infected sandflies from cases of human and canine kala-azar in Sicily and Malta. Experimental work was also undertaken in the Trans-Caspian region by CHODUKINE who infected sandflies by feeding them on dogs suffering from kala-azar and by SOFFIEWE and SCHEWTSCHENKO.

Susceptibility of Animals.

An observation of the greatest importance already referred to, from the point of view of experimental work, was the observation of SMYLY and

YOUNG (1924) that the Chinese hamster (*Cricetulus griseus*) was very susceptible to inoculation with *L. donovani*. Hitherto, though it had been possible to infect laboratory animals, particularly rats and mice, these animals were not very susceptible and frequently failed to become infected even when inoculated with large doses of the organisms. The discovery of the susceptibility of the hamster to kala-azar, like the discovery of the susceptibility of Asiatic monkeys to yellow fever, has removed one of the chief obstacles to experimental work on leishmaniasis. Following SMYLY and YOUNG's discovery it was found that various other small rodents were susceptible. YOUNG and LIU (1926) in China showed that in addition to the striped hamster (*C. griseus*), the giant hamster (*C. triton*), a vole of the genus *Microtus* and the house mouse (*Mus wagneri*) were markedly susceptible to inoculation with *L. donovani* and like the striped hamster exhibited little or no tendency to recovery. MAYER (1926) then demonstrated the susceptibility of the European hamster (*Cricetus frumentarius*) while BLANC and CAMINOPETROS (1930) proved this for the Macedonian spermophile (*Citillus citillus*) and ADLER and THEODOR (1931) for the Syrian hamster (*Cricetus auratus*) and the vole *Microtus g ntheri*. At the present time, therefore, there is no lack of susceptible animals for experimental work particularly as the Syrian hamster breeds readily in captivity. It has already been noted that owing to the susceptibility of the striped hamster to inoculation YOUNG and HERTIG (1926) examined 4,480 wild hamsters with the object of discovering if naturally acquired infections occurred. Of these one was found infected but as the animal had been kept in the same building as inoculated animals for 6 to 8 weeks before examination some doubt as to the accuracy of the observation is perhaps justifiable. It has been mentioned above that other animals may acquire infections naturally but dogs are the only ones that do so with any regularity. Where oriental sore occurs, as for instance in Bagdad, dogs are not infrequently found infected but as regards kala-azar it is well known that in the Mediterranean and Trans-Caspian kala-azar areas dogs frequently suffer from the disease, whereas in the Indian and Chinese areas for some unknown reason they escape. Though some observers claim to have demonstrated serological differences between the canine parasites and those of man these are not greater than differences between different strains of the human parasites so that it is safe to conclude that the parasites from man and the dog are identical. In the case of kala-azar in the Mediterranean and Trans-Caspian regions it has often been stated that dogs are a reservoir of the virus and it has been claimed that destruction of stray dogs has been followed by a fall in the incidence of the human disease. Whether the last statement is correct or not it is difficult to assert but it seems clear, in view of the fact that kala-azar maintains itself in India and China without any canine reservoir, that dogs in the Mediterranean and Trans-Caspian regions are not essential to the spread of the disease. It seems probable that a dog if it becomes infected is no more a reservoir than any human case.

Methods of Infecting Sandflies.

Experimental work with sandflies has been by no means an easy matter. It was difficult to keep these insects in captivity and often it was difficult to induce them to feed. Furthermore, for satisfactory experimental work it was necessary to have laboratory-bred insects and little was known about breeding them artificially. Intensive research has solved these problems so that it is now possible to have available laboratory-bred sandflies in which to study the development of leishmania and with which to make attempts at the transmission of these diseases.

Sandflies have been made to ingest leishmania directly from man by allowing them to feed on the skin of cases of kala-azar or on the surface of oriental sores. They have been fed on animals, particularly the hamster, mouse and dog, experimentally or naturally suffering from these diseases, while they have ingested cultures by feeding through membranes or by means of the method described by HERTIG and HERTIG (1927) which involves the insertion of the proboscis into a capillary tube containing liquid. By all these methods sandflies have become infected with the developmental forms of leishmania.

Alimentary Tract of Sandfly.

The alimentary tract of the sandfly (Fig. 1) is very similar to that of the mosquito. It commences at the proboscis, composed of the labrum-epipharynx above and the labium and hypopharynx below with the maxillæ and the mandibles.

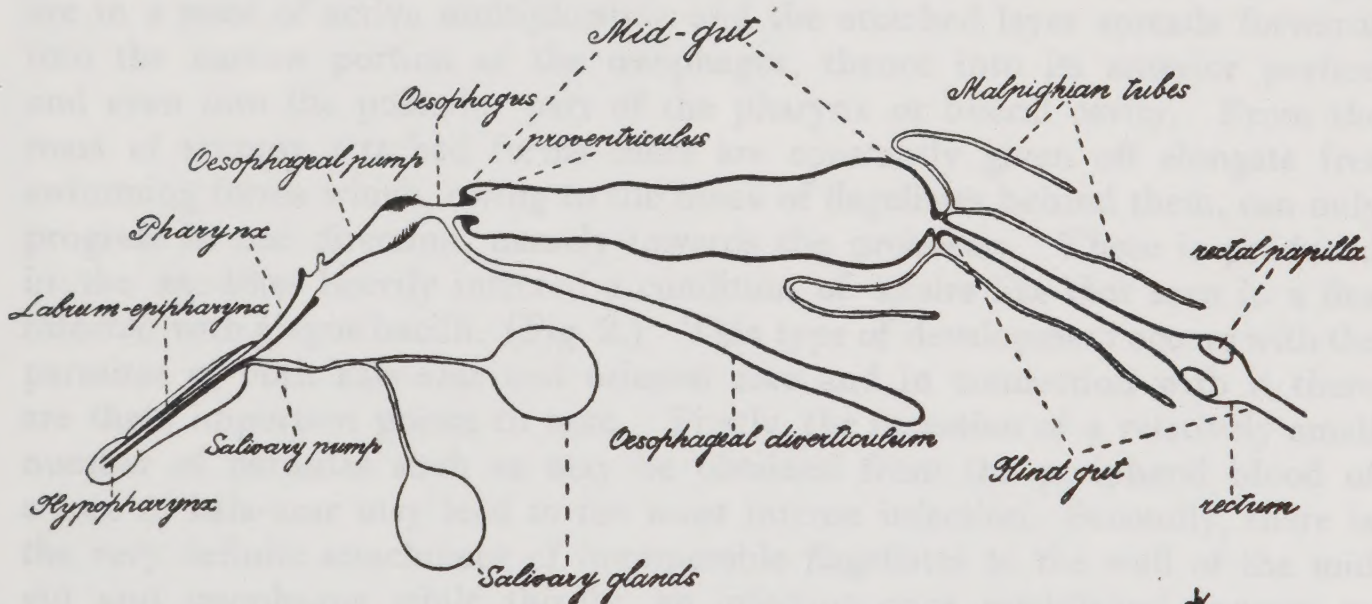


FIG. 1.—Alimentary tract of *Phlebotomus*.

This leads to the pharynx (often termed the buccal cavity). Following it is the œsophagus (often termed the pharynx) the anterior part of which is modified to form the chitinous pumping organ while near its posterior end is a bulbous dilatation armed with spines. A narrower continuation of the œsophagus

into which opens the diverticulum leads to the mid-gut into which it is invaginated to some extent to form the proventricular fold. The mid-gut is followed by the hind gut into which open at its anterior part the two ducts of the four malpighian tubes.

Development of Leishmania in the Sandfly.

The development of *L. donovani* in *P. argentipes* has been described in detail by SHORTT, BARRAUD and CRAIGHEAD (1926a); that of *L. tropica* in *P. papatasi* as observed by ADLER and THEODOR is essentially the same.

The leishmania ingested by feeding on man or an experimental animal enter the mid-gut or stomach with the blood and there during the next few days behave much as they do in the culture tube. The small leishmania forms increase in size and by the second day show signs of development of flagella. By the end of the second day or the beginning of the third day definite elongate flagellates are present. These multiply rapidly as do the earlier stages till finally in the stomach, particularly in its anterior part and in the proventricular folds, there are masses of flagellates rounded or elongate and often arranged in the characteristic rosettes. In certain cases in which the sandflies have been kept alive by subsequent feeds of blood the crowding of the flagellates towards the anterior part of the mid-gut is more marked, the whole of which including the proventricular folds becomes lined with stumpy forms attached to the surface, while in the lumen occur elongate forms, free swimming or in rosettes. The stumpy forms attached to the wall of the mid gut are in a state of active multiplication and the attached layer spreads forwards into the narrow portion of the œsophagus, thence into its anterior portion and even into the posterior part of the pharynx or buccal cavity. From the mass of stumpy attached forms there are constantly given off elongate free swimming forms which, owing to the block of flagellates behind them, can only progress in one direction, namely towards the proboscis. There is produced in the sandflies heavily infected a condition of affairs like that seen in a flea infected with plague bacilli. (Fig. 2.) This type of development occurs with the parasites of both kala-azar and oriental sore and in connection with it there are three important points to note. Firstly, the ingestion of a relatively small number of parasites such as may be obtained from the peripheral blood of a case of kala-azar may lead to the most intense infection. Secondly, there is the very definite attachment of innumerable flagellates to the wall of the mid gut and œsophagus while thirdly, an infection once established appears to persist for the remainder of the life of the sandfly. As explained above, these three features are characteristic of leptomonas of insects in general and indicate very strongly that the flagellate is in its natural host. Looking at the intensity of the infection in the sandflies and the presence of flagellates in the œsophagus and pharynx and even in the proboscis, it would seem inevitable that some would escape into the skin when such a sandfly feeds. (Fig. 3.) ADLER and THEODOR



FIG. 2.—Camera lucida drawing of median vertical section through head of *Phlebotomus argentipes* showing a massive infection with *Leishmania donovani* seven days after feeding. M., muscle; B.C., pharynx (buccal cavity); P., oesophagus (pharynx); F., plug of flagellates; M.G., midgut packed with flagellates; P.F., proventricular fold; C., chitin; S.G., salivary gland; C.S.D., common salivary duct; S.P., salivary pump; F.F., free elongate flagellates; C.P., crinkly portion of oesophagus (pharynx); J., junction between oesophagus (pharynx) and pharynx (buccal cavity); O., narrow posterior end of oesophagus. [After SHORTT, BARRAUD and CRAIGHEAD (1926). *Indian Journal of Medical Research*, xiii, 947.]

(1928, 1930b) have shown both for *L. tropica* and *L. donovani* that if infected sandflies are allowed to feed through a membrane on fluid, flagellates can be found in the fluid afterwards; observations which prove conclusively that flagellates actually do escape from the proboscis during the feeding act.

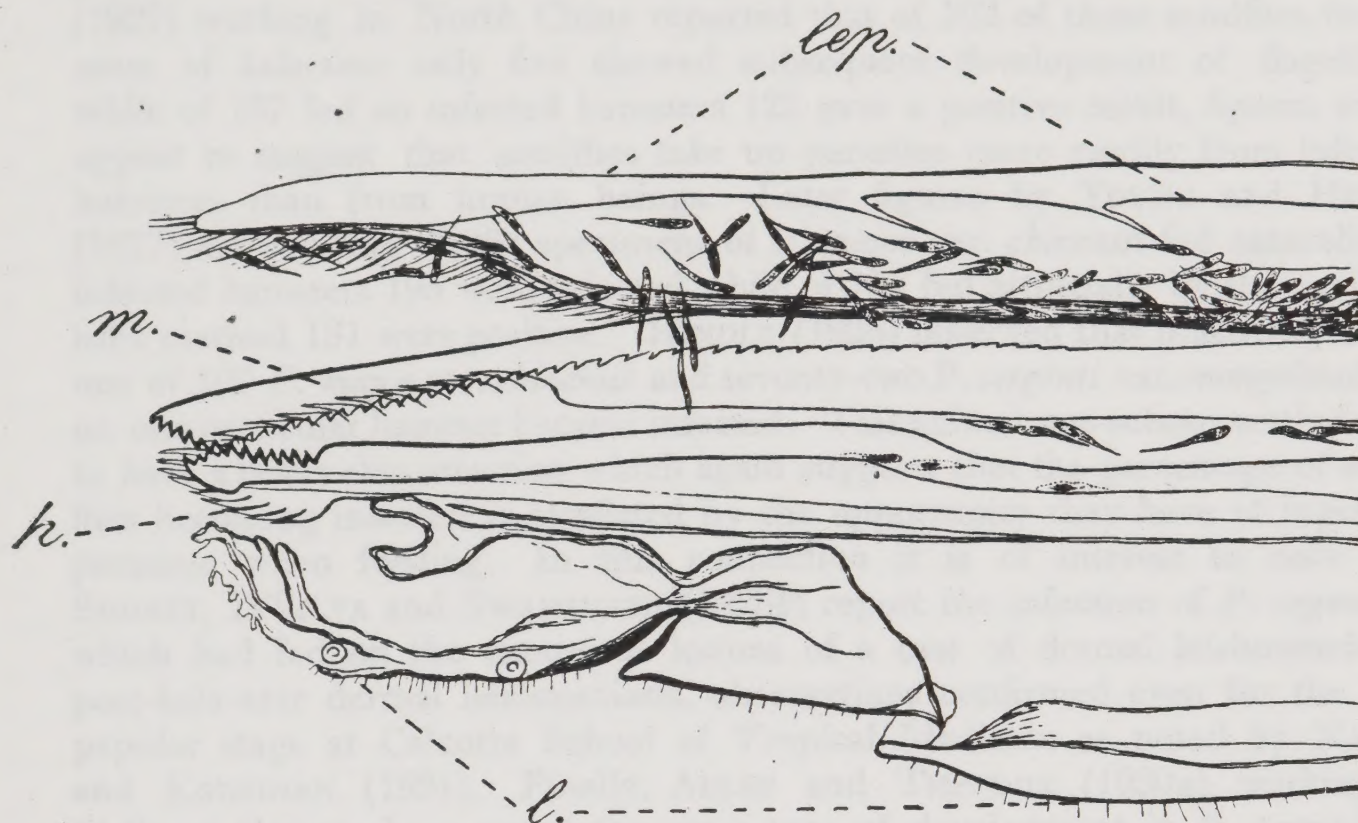


FIG. 3.—*Leishmania infantum* in food canal at end of proboscis of *Phlebotomus perniciosus*. lep., labrum epipharynx; m., mandibles; h., hypopharynx; l., labium; x, 750. (Drawing of preparation made by ADLER and THEODOR, in Catania.)

Percentage of Infections in Sandflies.

It must not be supposed that the development described occurs in all species of sandfly nor in all of any one species which have had the opportunity of feeding on a case of kala-azar in an experimental animal. Thus, in the first series of experiments carried out by KNOWLES, NAPIER and SMITH (1924) in Calcutta with *P. argentipes*, of fifty-six fed on cases of kala-azar, twenty-five became infected and of these only six had a heavy infection. CHRISTOPHERS, SHORTT and BARRAUD (1925), who confirmed these findings in Assam, found flagellates in five of thirteen sandflies which had fed but in only one were they very numerous. In another series of fourteen fed sandflies examined by cutting sections SHORTT, BARRAUD and CRAIGHEAD (1926) found infection of the mid-gut in ten, of which six had flagellates in the œsophagus also while four were entirely negative. In a still later communication these observers (1926b) report the examination of a sandfly on the ninth day in which the flagellates extended in an unbroken column from the proventriculus to the point where the biting appendages took origin, individual flagellates extending even further

forwards to a point anterior to the salivary pump. Following these experiments in India YOUNG and HERTIG (1926c) in North China found that of thirty-four *P. major* var. *chinensis* fed on hamsters infected experimentally with *L. donovani* twenty-nine were infected. The flagellates occurred chiefly in the anterior portion of the mid-gut and in the œsophagus. A little later PATTON and HINDLE (1927) working in North China reported that of 102 of these sandflies fed on cases of kala-azar only five showed subsequent development of flagellates, while of 157 fed on infected hamsters 122 gave a positive result, figures which appear to suggest that sandflies take up parasites more readily from infected hamsters than from human beings. Later figures by YOUNG and HERTIG (1927) showed that of 384 specimens of *P. major* var. *chinensis* fed naturally on infected hamsters 195 were infected while of 157 fed artificially by the capillary tube method 131 were positive. HINDLE (1928) observed that practically every one of 100 *P. major* var. *chinensis* and seventy-two *P. sergenti* var. *mongolensis* fed on one particular hamster became infected. This animal was subsequently found to have a heavy skin infection which again suggests that the percentage of sandflies becoming infected is regulated by the opportunity they have of ingesting parasites when feeding. In this connection it is of interest to note that SHORTT, D'SILVA and SWAMINATH (1928b) report the infection of *P. argentipes* which had fed on the cutaneous lesions of a case of dermal leishmanoid or post-kala-azar dermal leishmaniasis, observations confirmed even for the pre-papular stage at Calcutta School of Tropical Medicine as noted by NAPIER and KRISHNAN (1931). Finally, ADLER and THEODOR (1930a) working in Sicily at Catania have noted the same type of development in *P. perniciosus*. Of eighteen sandflies fed on hamsters experimentally infected with the local strain of *L. donovani* (*L. infantum*) fifteen were positive, while PARROT, DONATIEN and LESTOQUARD (1930) in Algiers showed that four of fifty-three of these sandflies collected from a kennel in which a dog suffering from kala-azar was housed were infected. In a later report ADLER and THEODOR (1931a) state that both *P. perniciosus* and *P. major* in Sicily gave a high percentage rate of infections when fed on infected hamsters. In one experiment in which thirteen *P. major* fed on an infected animal twelve became infected. These observers continued their investigations last year in Malta and ADLER has informed the writer that in certain batches of *P. perniciosus* fed on naturally infected dogs almost 100 per cent. became infected. This high percentage is of importance when considered in relation to the observations of ADLER and THEODOR (1931c) noted above that in infected dogs parasites occur throughout the skin.

Similar observations have been made in connection with oriental sore. ADLER and THEODOR (1926), who had previously (1925) discovered leptomonas in wild *P. papatasi* in Palestine and produced oriental sore in man by their inoculation into the skin, state that of 168 of these flies fed on an oriental sore sixteen became infected. PARROT and DONATIEN (1926, 1927) a little later reported the infection of *P. papatasi* after feeding on the tails of mice which had

developed lesions after inoculation with *L. tropica*. Of twenty-nine sandflies which were known to have fed on the same part of the tail eleven became infected. Feeding *P. papatasi* on cultures ADLER and THEODOR (1927, 1927a, 1927c) succeeded in readily infecting them with *L. tropica* but less so with *L. brasiliensis*. Of two strains of *L. infantum* one behaved like *L. brasiliensis* while the other resembled *L. tropica*, ninety-five sandflies becoming infected out of ninety-nine fed. ADLER and THEODOR (1928a) working in Bagdad and Mosul found that *P. sergenti* could be infected with *L. tropica* by feeding them with cultures or on oriental sores. Of seventeen sandflies fed on an oriental sore eleven became infected, the development of flagellates and their extension forwards towards the proboscis was as marked as in the case of *P. papatasi*. Meanwhile CHODUKIN (1928, 1928-29) in Tashkent succeeded in infecting unidentified sandflies by feeding them on cutaneous lesions of the dog, observations which were confirmed by SOFFIEWE and SCHEWTSCHENKO (1929) for *P. papatasi*. MILLS and MACHATTIE (1930) similarly showed that *P. papatasi* and *P. sergenti* could be infected with the canine strain of *L. tropica* in Bagdad. ADLER and THEODOR (1929a) have pointed out that different strains of *L. tropica* vary in their infectivity to sandflies. A strain from a village in Palestine, where during the preceding six years almost every inhabitant suffered from oriental sore, was highly infective to these insects, while a strain from Jericho, where the disease is comparatively rare, was much less so. HINDLE (1931) who, as already noted, had found that the North China strain of *L. donovani* developed very readily in *P. major* var. *chinensis*, has shown that an Indian strain and a Mediterranean strain do not develop so readily.

From the above summary of some of the records it is evident that in India *P. argentipes* can be readily infected with *L. donovani*, *P. major* var. *chinensis* with the strain of this parasite in North China and *P. perniciosus* with the human and canine Mediterranean strains (*L. infantum*), while *P. papatasi* and *P. sergenti* are easily infected with *L. tropica* in areas where oriental sore is endemic. In all these sandflies there is a tendency for the parasites to multiply rapidly, to attach themselves to the anterior part of the mid-gut and to spread forwards to the œsophagus, pharynx and even the proboscis. Furthermore it comes out that the number of sandflies which become infected in any batch is correlated with the chance they have had of taking up parasites, the extreme cases being those noted by ADLER and THEODOR and HINDLE, where close on 100 per cent. of flies became infected. It has also been noted that other strains of *Leishmania* as for instance *L. brasiliensis* do not develop so readily in these sandflies. Evidence has been obtained that *L. donovani* and *L. tropica* do not develop to any extent in other species of sandfly. YOUNG and HERTIG (1926a, 1927) found that in two sandflies of North China, *P. sergenti* var. *mongolensis* and *P. perturbans*, development was considerably less marked than in *P. major* var. *chinensis*, while NAPIER and SMITH (1927) noted that *L. donovani* in Calcutta would develop in *P. papatasi* but not to the same

extent that it did in *P. argentipes*. PATTON and HINDLE (1927) again found that in North China *P. major* var. *chinensis* was as a rule a better host than *P. sergenti* var. *mongolensis*. Thus, of 157 of the former fed on infected hamsters 122 were positive, while of 1,170 of the latter 430 were positive. The infections in *P. major* var. *chinensis* tended to extend forwards towards the pharynx while those in *P. sergenti* var. *mongolensis* were limited to the mid gut and usually died out in a few days. Similarly at Catania ADLER and THEODOR (1931a) have found that the parasite of infantile kala-azar produced an infection rate of 83·3 per cent. in *P. perniciosus* and one of only 0·75 per cent. in *P. papatasi*. These data indicate that, though there is a tendency for leishmania to develop in sandflies generally, certain species are much more suitable for active multiplication than others and it is only in them that the massive infections will occur with any regularity. There appears to be a specific relationship between certain species of sandfly and certain species of leishmania. In this connection it is of interest to note that ADLER and THEODOR (1930) have tested the behaviour of certain insect leptomonas in *P. papatasi*. For instance cultures of *Herpetomonas culicidarum*, *H. oncopelti*, *H. lygaeorum* and *H. muscidarum* fed to these insects by the capillary tube method persist in the mid-gut for periods varying up to thirteen days. They may pass into the hind gut but show no tendency to anterior migration or extension. There seems to be, therefore, a tendency for leptomonas to develop in any sandfly but again it is only in certain special cases that the intense development with attachment and extension forwards takes place.

Natural Infection in Sandflies.

In addition to the evidence that in the laboratory sandflies may be infected with leishmania there is that of the occurrence of natural infections in nature. The writer (1911), as already noted, recorded the occurrence of leptomonas in sandflies in Aleppo where oriental sore is endemic. ADLER and THEODOR (1925) reported the discovery of four *P. papatasi* naturally infected with *L. tropica* in Jericho. SHORTT, BARRAUD and CRAIGHEAD (1926c) discovered a naturally infected *P. argentipes* in a kala-azar house in Assam, while SHORTT, CRAIGHEAD, SMITH and SWAMINATH (1930) state that of a further 226 wild sandflies collected from kala-azar houses seven proved to be infected. PARROT and DONATIEN (1927) examined 181 *P. papatasi* in Biskra in Algeria in September, 1926, and found a single specimen infected with *L. tropica*. ADLER and THEODOR (1928a) again found in Mosul in Iraq a natural *L. tropica* infection in two *P. sergenti* out of 683 dissected. These observers (1931a) working in Catania examined 751 wild *P. perniciosus* and found infection in one which had been captured in a room in which a case of kala-azar was sleeping. In the same place 3,839 *P. papatasi*, 100 *P. sergenti* and 222 *P. major* were found to be uninfected. The discovery of four infected *P. perniciosus* by PARROT, DONATIEN and LESTOQUARD (1930) amongst fifty-three collected from a kennel, in which a kala-azar dog was kept has already been noted above.

Leishmaniasis of Lizards in Relation to Sandflies.

In addition to the evidence which points to a definite relationship between the human leishmania and sandflies evidence is accumulating that these insects are involved also in the spread of the leishmania of lizards referred to above. Certain species of *Phlebotomus* appear to nourish themselves almost entirely on lizards, particularly geckos. Thus, according to ADLER and THEODOR (1931a), *P. parroti* in Sicily feeds exclusively on geckos while the same is true to a large extent of *P. minutus*. It has been demonstrated by ADLER and THEODOR (1929) that *P. papatasi* becomes infected with a leptomonas if it feeds on an infected Bagdad gecko, *C. doriae*. The flagellates take up a position in the hind gut and attach themselves to its wall. They conclude that in Bagdad the gecko becomes infected by ingesting infected sandflies. This conjecture receives support from the recent work of SHORTT and SWAMINATH (1931) on a trypanosome (*Trypanosoma phlebotomi*) of an Indian gecko of the genus *Hemidactylus* which, as stated above, is liable to a leishmania infection also. The trypanosome develops in the intestine, eventually taking up a position in the hind gut. They have proved that laboratory-bred geckos may be infected by feeding them with infected sandflies. ADLER and THEODOR (1931a) in Sicily have similarly infected both *P. parroti* and *P. papatasi* with a trypanosome (*T. ptyodactyli*) of the gecko *T. mauritanica* and have discovered a naturally infected *P. parroti*. This gecko again is liable to a leishmania infection in North Africa. The leishmania infection was not discovered in the Sicilian gecko and they correlate this with the absence of *P. minutus*, which is present in North Africa. It is possible that the crithidia seen by YOUNG and HERTIG (1927) in *P. perturbans* in China which does not feed on man represent a trypanosome of a gecko or of a toad, on which amphibian the sandflies readily engorge themselves.

Virulence of Leishmanias in Sandflies.

Ample proof has been obtained that the flagellates which develop in sandflies after the ingestion of human parasites are capable of producing infections. The most striking instance is that reported by ADLER and THEODOR (1926a). Flagellates discovered in a wild *P. papatasi* in Jericho were inoculated by scarification into the skin of the arm of a volunteer. An oriental sore developed and laboratory-bred *P. papatasi* became infected when fed on it. With the flagellates from these sand flies oriental sore was again produced in a volunteer. SHORTT, BARRAUD and CRAIGHEAD (1927) infected one out of fifty-three mice inoculated intraperitoneally with flagellates from *P. argentipes* which had previously ingested *L. donovani*, similarly HINDLE and PATTON (1927, 1927a) demonstrated the infectivity of the flagellates which had developed in *P. sergenti* var. *mongolensis* in North China. YOUNG and HERTIG (1927) likewise infected hamsters with flagellates from *P. sergenti* var. *mongolensis* and *P. major* var. *chinensis*

by intraperitoneal inoculation, as also did HINDLE (1928). ADLER and THEODOR (1931) have infected a hamster by the intraperitoneal injection of flagellates from *P. perniciosus* in Catania. They (1927b) have produced some evidence in the case of *Leishmania tropica* by the cutaneous inoculation of volunteers that the flagellates which develop in *P. papatasi* are not infective till several days have elapsed from their first appearance in the sand flies. Attempts to infect hamsters by feeding them with the flagellates from sandflies or by rubbing the flagellates into scarified areas of the skin have so far been a failure.

Comparison of Insect Phases of Trypanosomes and Leishmania.

Before considering the actual transmission experiments it will be of interest to compare with the development of leishmania in sandflies that of certain trypanosomes in their transmitting hosts, for the analogy is very striking. It has already been explained above that the leptomonas of the flea inhabits the hind gut and is transmitted from flea to flea by rounded leishmania forms which are passed in the fæces and which are ingested by the flea larvæ. In species of *Drosophila* there may occur flagellates of the trypanosome type which have a similar life history. Rounded forms are passed in the fæces and these are ingested by the larvæ, leading to infection of the next generation of adults. In both these cases there is no question of any vertebrate host. In the case of *Trypanosoma lewisi* of the rat the flea abstracts trypanosomes from the blood and these undergo development in the intestine leading to heavy infections of the hind gut. Certain small trypanosomes known as metacyclic trypanosomes are voided in the fæces of the flea and if ingested by a rat will lead to its infection. Here the extra-corporeal stage, as far as the insect is concerned, is passed in the rat instead of on the ground. The same type of infection, as demonstrated by HOARE (1923, 1931), occurs in the case of the trypanosome of the sheep and crocodile, of which the sheep ked and the tsetse fly are the respective transmitting hosts. The observations of SHORTT and SWAMINATH (1931) on the trypanosome of *Hemidactylus* and its development in *P. argentipes* show that the same method of infection occurs in its case. It seems highly probable that this type of transmission of a vertebrate trypanosome is the primitive one and represents the first adaptation of a purely insect trypanosome to a vertebrate host. In the case of leptomonas there appears to be an analogy in the behaviour of *L. ceramodactyli* of the Bagdad gecko *C. doriae*. As already noted, it develops in *P. papatasi* producing a leptomonas infection of the hind gut and there is reason to suppose, though the experiment has not actually been carried out, that geckos become infected by devouring infected sandflies as occurs in the case of the gecko trypanosome just referred to. In the case of certain of the pathogenic trypanosomes of Africa, *T. congolense* and its allies transmitted by tsetse flies, it is well known that development of the ingested trypanosomes commences in the mid-gut and that the developmental forms extend forwards to the proboscis where massive infections occur and the infective forms are produced. In the

behaviour of the parasites of oriental sore and kala-azar in sandflies there is a parallel to the development of *T. congolense* in the tsetse fly. There is the initial multiplication in the mid-gut followed by extension forwards to the proboscis. The proboscis forms of *T. congolense* produce infections in susceptible animals when the tsetse fly feeds and by analogy it would be supposed that the proboscis forms of leishmania are the infecting agents and that oriental sore and kala-azar are transmitted by the bite of the sandfly.

Transmission Experiments with Sandflies.

Having arrived at the position that in endemic areas of kala-azar and oriental sore there are certain species of *phlebotomus* in which the local strains of leishmania develop to produce very heavy leptomonas infections of the mid-gut, œsophagus and pharynx; that these leptomonas leave the proboscis during the feeding act and that they are virulent in that they will produce oriental sore in man or kala-azar in animals when inoculated into the skin or intraperitoneally as the case may be, we have to turn our attention to direct transmission experiments in which infected sandflies are allowed to feed naturally. The type of development in the sandflies and the fact that the flagellates escape from the proboscis during feeding are very suggestive of infection taking place by way of the proboscis. Though this would appear evident it has to be admitted that, in spite of an enormous number of experiments, in only one instance in the case of oriental sore and one in the case of kala-azar is there any probability that infection has been experimentally conveyed by the bite of the sand fly. SHORTT, CRAIGHEAD and SWAMINATH (1928) reviewing the work of the Kala-Azar Commission in Assam state that 3,590 *P. argentipes* at the supposedly infective stage have been fed on white mice, 2,325 on Chinese hamsters and 1,247 on human volunteers without a successful result. In another paper SHORTT, CRAIGHEAD, SMITH and SWAMINATH (1930*b*) give some further indication of the extent of the work carried out in Assam. In all 273,467 *P. argentipes* were bred in the laboratory and 79,939 were actually fed. In spite of these discouraging results which at times led the members of the Commission to wonder if the whole of the remarkable development which takes place in the sandflies was merely a delusion and a snare from the point of view of indicating that they were transmitters of kala-azar in India, the experiments were continued. Finally SHORTT, SMITH, SWAMINATH and KRISHNAN (1931) were able to report that infection was produced in one hamster on which had fed during the course of seventeen months 144 *P. argentipes*, a large proportion of which must have been infected, as judged by dissection of samples after feeding. YOUNG and HERTIG (1927) report that in North China infected or probably infected sandflies were fed on 413 healthy hamsters without producing infection in a single one. Similarly HINDLE and PATTON (1927*a*) attempted to infect hamsters by infected sandflies in four ways. Sandflies were allowed to feed, flagellates from sandflies

were rubbed into the excoriated skin, sandflies crushed or intact were placed in the mouth and flagellates were inoculated intraperitoneally. As noted above, the only animals to become infected were four of a series of forty-six which had been inoculated intraperitoneally with flagellates from *P. sergenti*. ADLER and THEODOR (1927c) subjected three volunteers to the bites of ninety-two sandflies eighty-nine of which had previously fed on oriental sore and three on cultures of *L. tropica*. Of this total five were subsequently found to be infected but no infection of the volunteers resulted. In a later paper these authors (1929a) describe further experiments in which *P. papatasi* to the number of two to eighty-eight, infected by feeding on cultures of *L. tropica*, were allowed to feed on twelve volunteers and one puppy. A negative result was obtained in all except one volunteer on whose arm after ten months there appeared on the area on which the sandflies had fed lesions containing leishmania. As this volunteer had lived in a quarter of Jerusalem in which a number of imported cases of oriental sore can always be found and as six months after the feeding inoculation experiments were performed on both arms, but at points distant from the area on which the feeding had been carried out, the authors are reluctant to consider the experiment conclusive, even though the lesions appeared at the actual site of feeding. Finally there is the experiment of CHODUKIN and his co-workers (1931) mentioned at the beginning of this paper. Here wild sandflies (*P. papatasi* and *P. sergenti*) were introduced during three months into a cage in which were housed at one end a dog suffering from cutaneous leishmaniasis and at the other four healthy puppies. One of the puppies, the only one which survived, was seen about two months after the three months' period to have lesions on the snout in which leishmania were demonstrated. This experiment is vitiated to some extent by the fact that the puppy was of local origin and might conceivably have been incubating the disease before the experiment commenced. Furthermore, though it is stated that all the animals were carefully cleared of fleas before the experiment commenced it seems possible that some fleas or other ectoparasites were present. It has already been shown above that infection may spread amongst animals kept in close association with one another.

CONCLUSION.

All the experiments described above have been attempts to transmit kala-azar or oriental sore by the bite of infected sandflies and it must be admitted that the hopes raised by the discovery of the remarkable development of leishmania in sandflies have not been realised. It was supposed that given a susceptible animal transmission by the bite would be easily accomplished. This has not been the case, so that if the theory of sandfly transmission of these diseases is not to be abandoned, which it seems unreasonable to do, it has to be assumed that some fundamental factor in all these experiments has been omitted or that infection may take place otherwise than by the bite. ADLER

(1929), in an analysis of the leishmania sandfly problem, lays stress on the fact that in the Mediterranean area very young children and dogs suffer from kala-azar whereas in India and North China the human beings who contract the disease are generally older while dogs are not infected. He sees in this an indication that in India and North China infection may be acquired by the crushing of infected sandflies on the skin whereas in the Mediterranean area the method of infection is by the bite, as very young children and dogs are unlikely to crush these insects. Similarly in Bagdad human beings and dogs commonly suffer from oriental sore while in Palestine infection in dogs does not occur. The inference is that in Bagdad infection is by the bite and in Palestine by crushing. In support of this it is stated that the development of *L. tropica* is more marked in *P. sergenti* of Bagdad than in *P. papatasi* of Palestine. KNOWLES (1927) has suggested that in endemic areas in India there may be very extensive inoculation of the population with leishmania by the sandfly but that in only certain cases of lowered resistance will infection take place, as for instance after typhoid fever or malaria. Whatever may be the explanation of the difficulties encountered in effecting transmission by the sandfly, there is abundant evidence, apart from the specific development of the parasite in these insects, that they are associated with the diseases. In India it was SINTON's observation that the distribution of *P. argentipes* in India coincided with that of kala-azar that led KNOWLES, NAPIER and SMITH to make the outstanding discovery in connection with its transmission. NAPIER (1925) in a study of environment associated with kala-azar in Calcutta concluded that the evidence was in favour of the sandfly being the transmitter of the disease, while more recently the same observer (1931) in an epidemiological investigation of kala-azar in a rural area in Bengal found that all the data secured fitted into the hypothesis that *P. argentipes* was the vector. In North China again kala-azar is limited almost entirely to areas north of the Yangtse and nothing is known of sandflies south of this river. MORRIS (1931) has reported one case from Sungkiang fifteen miles south west of Shanghai. ADLER and THEODOR (1931*b*) point out that in Italy the distribution of *P. perniciosus* and kala-azar are identical. In Catania it is significant that both this sandfly and kala-azar are rare at the centre of the town while the absence of the sandfly and kala-azar above a certain altitude even in the centre of endemic foci is in favour of this particular species being the vector. As regards oriental sore wherever it occurs sandflies are common though it cannot be asserted that the converse is true. Epidemiological evidence such as this could be multiplied but though much of it does not definitely incriminate the sandfly in no case can it be said to have refuted the theory of sandfly transmission.

It may be that infection results from the ingestion of infected sandflies or the crushing of them on the skin but neither of these methods would account for the anterior development, which is much more suggestive of infection through the bite. In the light of NAPIER and KRISHNAN's (1931) statements

regarding dermal leishmaniasis in Bengal it seems possible that in kala-azar, as also in oriental sore, a primary local skin infection, perhaps not detectable, may be produced by the bite of an infected sandfly and that in only certain cases or when the parasite is particularly virulent is natural kala-azar produced by generalisation of the infection. Row (1912) proved that in the monkey a purely local lesion might result from the inoculation of *L. donovani* in the skin, while KORKE (1914) confirmed this observation but pointed out that in some cases a generalised infection might result and in others both a local lesion and a generalised infection. SHORTT and SWAMINATH (1926) inoculated a monkey intradermally with *L. donovani* and discovered parasites in the internal organs one year later.

It is evident that the problem has not yet been completely solved but the fact that in one or two cases transmission by the bite of the sandfly has been effected, that there is undoubtedly a specific relationship between leishmania and these insects in the light of the remarkable development that takes place, that the flagellates in the sandfly are virulent and are known to escape from the proboscis during feeding, afford overwhelming evidence that sandflies are responsible for the spread of the two diseases kala-azar and oriental sore.

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DISCUSSION.

Lieut.-Colonel Sinton said that his interest in sandflies had led him to investigate the question of their specific distribution in relation to kala-azar and oriental sore. It is well known that a line joining Bombay and Simla almost exactly separates the recorded distribution of these two diseases in India. Our present knowledge of the distribution of *P. argentipes* shows it as confined to the east of this line, except for a small focus in Kathiawar, while *P. sergenti* is only recorded to the west. This distribution has suggested that the two insects may be the carriers of Indian kala-azar and oriental sore respectively. It is significant that the local names for cutaneous leishmaniasis in India, such as Delhi boil, Frontier sore, Sind sore, Lahore sore, etc., are the names of areas from which the records of *P. sergenti* are most common.

The SERGENTS and ADLER have collected considerable evidence to incriminate *P. papatasi* as the carrier of oriental sore in the Mediterranean area. The latter author has found reason to believe that *P. sergenti* may also act as a carrier

in more eastern regions. There seems to be no relationship between the distribution of this disease and *P. papatasi* in India.

The similarity between the recorded distribution of *P. argentipes* and Indian kala-azar (Sinton, 1924)* between *P. sergenti* and oriental sore (SINTON, 1922)†, between *P. perniciosus* and infantile kala-azar (SINTON, 1925)‡ and *P. chinensis* and Chinese kala-azar (SINTON, 1925) is suggestive. The evidence which has accumulated in the last decade supports these theories.

Although the epidemiological and experimental evidence seems to be almost conclusive in incriminating the sandfly as the transmitting agent of leishmaniasis, both visceral and cutaneous, yet it seems to me that we have not yet proved beyond all shadow of doubt that this is the usual or only method of carriage of these diseases.

Dr. Edward Hindle said that he considered the evidence in support of sandflies being concerned in the transmission of leishmania was so striking as to render it almost a waste of time to look elsewhere. He put it as strongly as that, for if one summarised the positive facts in support of this view, the negative fact of the difficulty in producing actual transmission under laboratory conditions was quite outweighed. He was unable to understand why there seemed to be such reluctance to accept the sandfly hypothesis, for he was not aware of a similar scepticism being extended to the transmission of filariasis by mosquitoes, although he did not know that any actual transmission experiments with this infection had been recorded. In both cases there was strong epidemiological evidence in support of the theory that a particular insect, or group of insects was concerned in the transmission; in both cases there was a specific development in the insect host; and in both cases the actual method of transmission was the result of inference rather than direct experiment. With reference to leishmania in China, he had found evidence of the existence of distinct strains of the parasite. In addition the leishmania of Chinese kala-azar seemed to be distinct from that of Indian kala-azar, judging from its development in sandflies. Examples of *Phlebotomus chinensis*, the sandfly in which a specific development of the Chinese strain takes place, were fed on hamsters infected with an Indian strain, and although flagellation occurred in these sandflies, there was no attachment of the flagellates to the lining of the gut, which attachment is an invariable feature when *P. chinensis* is infected with Chinese strains of leishmania. Apart from that, although the Chinese leishmania will develop to the flagellate stage in the three species of *Phlebotomus* occurring in North China (one of which feeds on reptiles and batrachians and never bites man), there is a marked difference in one respect. In *P. chinensis* the parasites not only multiply but become attached to the lining of the gut, then grow forward,

*Sinton, J. A. (1922). *Ind. J. Med. Res.*, ix (3), 579.

†Sinton, J. A. (1924). *Ibid*, xi (4), 1045.

‡Sinton, J. A. (1925). *Ibid*, xii (4), 726.

and eventually may extend into the proboscis. He had seen under the microscope specimens of Chinese sandflies in which the flagellates were escaping from the tip of the proboscis. It was a curious fact that when numbers of these infected flies were fed on hamsters no infection was obtained, but it was worth remembering that the susceptibility of the hamster to infection with flagellates was by no means as great as was sometimes believed. In his experiments only about one hamster in twelve became infected after the intraperitoneal injection of flagellates, obtained by grinding up the guts of infected sandflies, and in addition the injection of flagellates subcutaneously was approximately ten times less efficient than the intraperitoneal route. Consequently, even if all the flagellates in the insect were injected into the subcutaneous tissues when it fed, the odds against infection being produced in the hamster by the bite of an infected sandfly was less than one in a hundred.

Another point to which he had not paid sufficient attention whilst in China was the possibility that leishmaniasis is essentially a cutaneous infection. On returning to this country, and examining his material, he had found hamsters which showed no trace of visceral infection yet had a good cutaneous infection. Conceivably many of the supposedly negative hamsters in his transmission experiments might have been found positive if the skin had been examined. It was a point to be borne in mind when making transmission experiments that parasites should be looked for in this site.

Epidemiological evidence to incriminate the sandfly in China was quite as clear as in India. Sandflies did not occur south of the Yangtze and kala-azar also had not been found south of the Yangtze. He had perhaps repeated some of the evidence given by Dr. WENYON, but he wished to emphasize his opinion that the evidence for the sandfly being responsible for the transmission of kala-azar was so strong that it was better to concentrate attention on transmission experiments with them; rather than to leave the sandfly and enter upon the study of other insects against which the evidence was not so strong.

Dr. H. J. Smyly said he thought that the evidence as it had been presented was almost conclusive that the sandfly was the transmitting agent of kala-azar. But from the work of SHORTT and others he felt that until the final proof of transmission by the sandfly had been brought forward the mind should be kept open as to the possibility of infection through the alimentary route. There were certain points which lent colour to this view which had been mentioned by Dr. WENYON. One which he thought he did not mention was that both SHORTT and also KHAW in Peking had found a high percentage of infections on feeding hamsters with flagellates by the mouth. There was, of course, a difficulty in imagining how such a delicate organism could survive outside the body so as to produce an alimentary infection, unless, indeed, the patient got the infection by ingestion of infected sandflies themselves. The infection of specific sandflies with adhesion to the gut wall and the forward growth into the proboscis was certainly strong circumstantial evidence, and SHORTT's

experimental infection of a hamster from the bites of infected sandflies proved that possibility. On the other hand the enormous number of negative experiments, including those on man, showed that there was some factor not yet discovered which was still a matter of conjecture. There were other diseases which were only contracted when some depressing circumstance lowered the resistance to a given organism, and there might be some crucial factor of that kind here which had not yet been discovered.

Professor J. G. Thomson said that the able, highly critical and exhaustive review of Dr. WENYON on the present position of our knowledge regarding the transmission of leishmaniasis strongly incriminated various species of sandflies as the possible vectors. It was clear, however, that some factor or factors governing the successful experimental infection of man and susceptible animals were unknown. Until positive and definite results regarding the transmission were obtained it was impossible to feel confident that this problem had been solved beyond all doubt.

Wing-Comdr. H. E. Whittingham referred to attempted leishmania transmission experiments with sandflies bred in captivity in England, in 1923. (See Report on Health of the R.A.F., 1923, p. 68). Two batches of fifty each were fed on two cases of oriental sore, one from India, the other from Egypt. The oriental sore from India was ulcerated, and the sandflies were fed on the edge of the sore. The other from Egypt was an unbroken sore. After twenty-four hours the sandflies were allowed to feed daily on three volunteers for sixteen days in two cases and seventeen in the other. In no instance did an oriental sore develop. Two of the three volunteers remained in this country, but the other went out to Mesopotamia, and still no oriental sore developed. He was inclined, however, to agree with the majority of the speakers, that the phlebotomus was most probably the vector of this disease, but it seemed rather difficult to correlate the fact that there were myriads of sandflies and so few cases of leishmaniasis. If, however, the habits of the sandfly were studied the matter became plainer. Sandflies dislike getting wet, and that is the reason why they will not bite a person when one is sweating freely or where there is a broken sore. The second point was that seven days were needed at least for the completion of the cycle of leishmania in the phlebotomus. The average sandfly died in childbirth about the fourteenth or sixteenth day, during which period it fed probably on the first day of its life, again on about the fourth or fifth, and again on about the eighth or ninth, and that usually was its last feed. Unless it bit an infected person in its first feed, obviously it could not infect anybody with leishmaniasis. Perhaps these factors help to explain why there were so few cases of oriental sore and so many sandflies. Furthermore, the female phlebotomus, having entered a human dwelling and fed on man, usually remains domestic, thus accounting for the localised geographical distribution of leishmaniasis.



